

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

IN THE SPECIFICATION

Please amend the Specification as follows:

Please replace the paragraph beginning on page 12, line 21 with the following paragraph:

Figure 12 is a schematic representation of the sequence of the human  $\beta$ -globin gene (GenBank locus HUMHBB; SEQ ID NO.:1) and of several primers described herein.

Please replace the paragraph beginning on page 13, line 7 with the following paragraph:

Figure 13 is the sequence of Human Growth Hormone Sequence (SEQ ID NO.: 15) Example 7. The relative positions of the oligonucleotides used in the experiment are underlined. Oligonucleotide GH1 hybridizes to the complement of the Human Growth Hormone sequence shown.

Please replace the paragraph beginning on page 34, line 1 with the following paragraph:

A template with or without a single nucleotide base containing a 1,3 propane diol moiety (designated as non-replicable element "X") and a primer complementary to the 3' end of the template were synthesized in order to demonstrate the ability of the 1,3 propane

diol moiety to serve as a non-replicable element and halt DNA synthesis. The sequences synthesized are as follows:

<u>Name</u>	<u>Sequence (5' -&gt; 3')</u>	<u>SEQ ID NO.</u>
DNA II 207	GCTCCCTTAGCATGGGAGAGTCTCCGGTTC	1
DNA II 207X	GCTCCCTTAXCATGGGAGAGTCTCCGGTTC	2
P12	GAACCGGAGACT	3

The primers and templates were annealed to form the following primer template complexes:

5' GCTCCCTTAGCATGGGAGAGTCTCCGGTTC	<u>SEQ ID NO.:1</u>
TCAGAGGCCAAG5'	<u>SEQ ID NO.:3</u>

5' GCTCCCTTAXCATGGGAGAGTCTCCGGTTC	<u>SEQ ID NO.:2</u>
TCAGAGGCCAAG5'	<u>SEQ ID NO.:3</u>

The primer template complexes were then extended with various DNA polymerase (Perkin Elmer - Cetus Corp.), BST polymerase I, AmpliTaq polymerase (Bio-Rad Laboratories, Hercules, CA) and Sequenase polymerase (United States Biochemical, Cleveland, OH) in the presence of  $\alpha$ -[32P]-dCTP and the products were subjected to electrophoresis on a denaturing polyacrylamide gel.

Please replace the paragraph beginning on page 35, line 17 with the following paragraph:

Four oligonucleotides were prepared, two of which contained the non-replicable 1,3-propane diol moiety. The sequences were synthesized on an Eppendorf Ecosyn D300, automated DNA synthesizer with the MMT-propane diol phosphoramidite in the 3' position. When

entering the sequence into the synthesizer, a Z was introduced. The sequences are presented in the following table:

Name	Sequence (5' -> 3')	SEQ ID NO.:
BGP-2 22	GGGTGGGAAAATAGACCAATG	4
BGP-2 22X	GGGTGGGAAAATAGACC <del>X</del> ATG	5
BGP-1 30	GGCAGGAGCCAGGGCTGGGCATAAAAGTCA	6
BGP-1 30X	GGCAGGAGCCAGGGCTGGGCATAAA <del>X</del> GTCA	7

Please replace the paragraph beginning on page 36, line 4 with the following paragraph:

The sites of complementarity of the oligonucleotides in the human  $\beta$ -globin gene (GenBank Locus HUMHBB; SEQ ID NO.:1) is shown in Figure 12.

Please replace the paragraph beginning on page 38, line 8 with the following paragraph:

The products of the reactions (3  $\mu$ l) were mixed with 10  $\mu$ l 4N NaOH, 250 mM EDTA and were blotted onto a Zeta-Probe membrane (Bio-Rad Laboratories, Hercules, CA). Also included on the membrane was 56, 118 and 231 ng of the plasmid pHB<sup>A</sup> similarly denatured. The membrane was hybridized with 5'-<sup>32</sup>P-CTGCAGTAACGGCAGACTTCTCCT (SEQ ID NO.:8) at 55°C for three hours in 5X SSPE, 1% SDS, 5 mg/ml Blotto, 10  $\mu$ g/ml Homomix I RNA. After hybridization the blot was washed at room temperature in 6X SSC and then scanned in a Bio-Rad Molecular Imager. The reaction produced approximately 250 fold amplification demonstrating the process of the present invention results in the amplification of the nucleic acid sequence of interest.

Please replace the paragraph beginning on page 39, line 15 with the following paragraph:

To control the size of the fragments generated by the amplifications, two reactions were performed. One reaction contained Thermus aquaticus polymerase buffer, 5 pmol of primers BGP5-22X and BGP4-22X,  $1 \times 10^8$  molecules of plasmid pH $\beta^A$ , and 3 units of Ampli-Taq Polymerase. The second control included the same ingredients as the previous reaction except that the primers BGP1-35X and BGP2-35X were used. The template DNA was denatured for 4 min at 94°C and then cycled 48 times using the following conditions program: annealing and polymerization at 55°C for 30 sec; denaturation at 94°C for 30 sec. At the end of the last cycle the samples were annealed at 55°C for 30 sec and polymerized at 72°C for 4 min.

<u>Primer</u>	<u>Sequence (5' → 3')</u>	<u>SEQ ID NO.:</u>
BGP1-35X	CCAGGGCTGGGCATAAAAGTCAGGGCAGAGXCATC	9
BGP2-35X	GGGTGGGAAAATAGACCAATAGGCAGAGAGXGTCA	10
BGP4-22X	CCAAAGGACTCAAAGAAXCTCT	11
BGP5-22X	CCTCACCCCTGTGGAGCCXCACC	12

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Please replace the paragraph beginning on page 40, line 8 with the following paragraph:

The entire reaction (15  $\mu$ l) was mixed with 1.6  $\mu$ l 10X Ficoll loading buffer and subjected to electrophoresis in a 1.5% agarose gel (Bio-Rad ultrapure agarose). Electrophoresis was performed in TBE buffer for 90 min at 110 volts. The gel was subsequently stained with ethidium bromide (1  $\mu$ g/ml) for 30 min, destained for 15 min, and photographed by ultraviolet (UV) illumination. The electrophoresed DNA was then transferred to a nylon membrane (Zeta probe, Bio-Rad) by alkaline transfer (Reed, K. C. and D. A. Mann, Rapid transfer of DNA from agarose gels to nylon membranes, Nucleic Acids Res. 13:7207-7221 (1985)) and fixed to the membrane by UV radiation (Church, G. M. and W. Gilbert, Genomic Sequencing, Proc. Natl. Acad. Sci. U. S. A. 81:1991-1995 (1984)). The membrane was prehybridized in 5X SSPE, 1% SDS, 10  $\mu$ g/ml homomix RNA and 0.5% dehydrated powdered skim milk (Carnation, Los Angeles, CA) for one hour and subsequently hybridized with 2.5 x 10<sup>6</sup> cpm/ml 5' end <sup>32</sup>P labeled probe 5'CAGGAGTCAGGTGCACCATGGT (SEQ ID NO.:13) for two hours at 55°C. The membrane was washed twice with 6X SSC for 30 min at room temperature and autoradiographed at room temperature for 30 min.

Please replace the paragraph beginning on page 42, line 4 with the following paragraph:

Two fold serial dilutions of the LLA and PCR products (1/64  $\mu$ l, 1/32  $\mu$ l, 1/16  $\mu$ l, 1/8  $\mu$ l, 1/4  $\mu$ l, 1/2  $\mu$ l, 1  $\mu$ l, 2  $\mu$ l, 4  $\mu$ l, and 8  $\mu$ l) were mixed with 1x Ficoll loading buffer and subjected to electrophoresis in a 1.5% agarose gel. Electrophoresis was performed in 1x TBE buffer for 90 min at 110 volts. The electrophoresed DNA was transferred to a nylon membrane by alkaline transfer (Reed and Mann, 1985), cross linked by UV radiation (Church and Gilbert, 1984), and then neutralized with 2x SSC. Subsequently, the membrane was hybridized in 5x SSPE, 1% sodium dodecyl sulfate (SDS), 10  $\mu$ g/ml homomix RNA, 0.5% powdered skim milk and  $2.5 \times 10^6$  cpm of the  $^{32}$ P labeled probe 5' CAGGAGTCAGGTGCACCATGGT (SEQ ID NO.:13). The hybridization took place at 55°C for two hours. After hybridization, the membrane was washed twice with 6x SSC at room temperature for 15 min. and then scanned and quantified with the Bio- Rad GS-250 Molecular Imager.

Please replace the paragraph beginning on page 43, line 11 with the following paragraph:

The 15  $\mu$ l reaction volumes were mixed with 1.6  $\mu$ l 10X Ficoll loading buffer and subjected to electrophoresis in a 1.5% agarose gel (Bio-Rad ultrapure agarose). Electrophoresis was

performed in 1x TBE buffer for 90 min at 110 volts. The gel was subsequently stained with ethidium bromide (1  $\mu$ g/ml) for 30 min, destained for 15 min, and photographed by ultraviolet (UV) illumination. The electrophoresed DNA was then transferred to a nylon membrane by alkaline transfer (Reed and Mann (1985)) and fixed to the membrane by UV radiation (Church (1984)). The membrane was prehybridized in 5X SSPE, 1% SDS, 10  $\mu$ g/ml Homomix RNA and 0.5% dehydrated powdered skim milk for one hour and subsequently hybridized with  $2.5 \times 10^6$  cpm/ml of the 5'-P<sup>32</sup> labeled probe 5' CAGGAGTCAGGTGCACCATGGT (SEQ ID NO.:13) for two hours at 55°C. The membrane was washed twice with 6X SSC for 30 min at room temperature and the reactions were quantified with the Bio-Rad GS-250 Molecular Imager.

Please replace the paragraph beginning on page 44, line 16 with the following paragraph:

Oligonucleotides and ribonucleoside containing oligonucleotides of the following sequences were prepared using standard automated solid-phase synthesis methods. The uracil nucleoside in oligonucleotides GH1 and GH2 are ribonucleosides.

<u>Name</u>	<u>Sequence (5' <math>\rightarrow</math> 3')</u>	<u>SEQ ID NO.:</u>
GH1 SEQ	TTCCCAACCAUTCCCTTA	14

GH2 SEQ	GGATTTCTGUTGTGTTTC	15
GH3 SEQ	TTCCCAACCATTCCTTA	16
GH4 SEQ	GGATTTCTGTTGTGTTTC	17
Md114	TAGCGTTGTCAAAAAGCC	18

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Please replace the paragraph beginning on page 47, line 19 with the following paragraph:

LLA primers containing the non-replicable propanediol element ("X") were synthesized. Primers flank the  $\beta^s$  (20A>T) and  $\beta^c$  (19G>A) mutation of the human  $\beta$ -globin gene (see Figure 14).

Primer sequences (5' -> 3') are as follows:

UP 8	TAAGCCAGTGCCAGAAGAGCCAAXGAC	SEQ ID NO.:19
UP 7	TACGGCTGTCATCACTTAGACXTCA	SEQ ID NO.:20
UP 6	CCCTGTGGAGCCACACCCTAGXGTT	SEQ ID NO.:21
UP 5	AATCTACTCCCAGGAGCAGGGXGGG	SEQ ID NO.:22
UP 4	GAGCCAGGGCTGGGCATAAAAXTCA	SEQ ID NO.:23
UP 3	GGCAGAGCCATCTATTGCTTAXATT	SEQ ID NO.:24
UP 2	TGCTTCTGACACAACCTGTGTTXACT	SEQ ID NO.:25
UP 1	AGCAACCTCAAACAGACACCAXGGT	SEQ ID NO.:26
LP 2	CCTCACCACCAACTTCATCCAXGTT	SEQ ID NO.:27
LP 3	AACCTTGATACCAACCTGCCCXGGG	SEQ ID NO.:28
LP 4	TATTGGTCTCCTTAAACCTGTXTTG	SEQ ID NO.:29
LP 5	TTCTCTGTCTCCACATGCCAXTTT	SEQ ID NO.:30
LP 6	CAGTGCCTATCAGAAACCCAAXGAG	SEQ ID NO.:31
LP 7	AAATAGACCAATAGGCAGAGAXAGT	SEQ ID NO.:32



LP 8	GACCACCAGCAGCCTAAGGGTXGGA	SEQ ID NO.:33
LP 9	TTCCTATGACATGAACTTAACXATA	SEQ ID NO.:34
LP 10	AAACTGTACCCTGTTACTTCTXCCC	SEQ ID NO.:35
LP 11	CAATCATTCGTCTGTTTCCCAXTCT	SEQ ID NO.:36

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Please replace the paragraph beginning on page 49, line 5 with the following paragraph:

To compare LLA and PCR efficiencies, PCRs were performed using the following primers (5' -> 3') corresponding to UP 8, LP 8 and LP 11 but without the non-replicable propanediol element "X":

UP 8-x	TAAGCCAGTGCCAGAAGAGCCAAGGAC	SEQ ID NO.: 37
LP 8-x	GACCACCAGCAGCCTAAGGGTGGGA	SEQ ID NO.: 38
LP 11-x	CAATCATTCGTCTGTTTCCCATTTCT	SEQ ID NO.: 39

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Please replace the paragraph beginning on page 49, line 18 with the following paragraph:

PCR and LLA amplification products were labeled in a primer extension reaction using the 5'-biotinylated primer MD 792 (5' CACCTTGCCCCACAGGGCAGTAACG 3' (SEQ ID NO.:84); see Figure 14). A 2  $\mu$ l-aliquot of the LLA or PCR reaction was mixed with 18  $\mu$ l of a mixture containing 10 pmoles MD 792, 10 mM Tris-HCl pH 9.2, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP's and 1 U AmpliTaq DNA polymerase. Reactions were placed in the thermal cycler and subjected to one cycle of heating and cooling as follows: 94°C for 2 minutes, 55°C for

2 minutes, 72°C for 5 minutes.

Please replace the paragraph beginning on page 53, line 1 with the following paragraph:

Eighteen Factor V gene specific primers, each containing the non-replicable propanediol element near the 3'-end, were synthesized. The primers flank the Factor V Leiden mutation (1691 G>A) site in exon 10. The 5' to 3' sequences are shown below:

FVU9	AGCACACCAACATGACACATGXATA	SEQ ID NO.:40
FVU8	CACATGTACCCTAGAACTTAAAGXATA	SEQ ID NO.:41
FVU7	AATTGGTTCCAGCGAAAGCXTAT	SEQ ID NO.:42
FVU6	CAGGCAGGAACAACACCATXATC	SEQ ID NO.:43
FVU5	AGAGCAGTTCAACCAGGGGXAAC	SEQ ID NO.:44
FVU4	TCTTAGAGTTTGATGAACCCAXAGA	SEQ ID NO.:45
FVU3	AAATGATGCCCAGTGCTTAACXAGA	SEQ ID NO.:46
FVU2	CCATACTACAGTGACGTGGACXTCA	SEQ ID NO.:47
FVU1	GAGAGACATCGCCTCTGGGXATA	SEQ ID NO.:48
FVL1	TTCTAGCCAGAAGAAATTCTCXGAA	SEQ ID NO.:49
FVL2	TTATTTAGCCAGGAGACCTAAXATG	SEQ ID NO.:50
FVL3	CTGTTCTCTTGAAGGAAATGCXCCA	SEQ ID NO.:51
FVL4	GGTGCTAAAAAGGACTACTTGXCAA	SEQ ID NO.:52
FVL5	CTTCGGCAGTGATGGTACTGAXAAA	SEQ ID NO.:53
FVL6	AACAGACCTGGAATTTGAAACXAAG	SEQ ID NO.:54
FVL7	CAACTTGCTCAACACATCCAAXACC	SEQ ID NO.:55
FVL8	AAGGAAGAAATTAGGAAAGGCXAAT	SEQ ID NO.:56
FVL9	CATTTTtaggagggttattaccxatt	SEQ ID NO.:57

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Please replace the paragraph beginning on page 54, line 8 with the following paragraph:

To label the amplification products, a 4  $\mu$ l-aliquot of the LLA reaction was mixed with 38  $\mu$ l of a mixture containing 10 pmoles of 5'-biotinylated MD 792, 10 pmoles of 5'-biotinylated FVU0 (5' CTACTTCTAATCTGTAAGAGCAGA 3'; SEQ ID NO.:58), 10 pmoles of biotinylated FVL0 (5' GAAAGGTTACTTCAAGGACAAA 3'; SEQ ID NO.:59), 10 mM Tris-HCl pH 9.2, 50 mM KCl, 2.5 mM  $MgCl_2$ , 0.2 mM dNTP's and 2 U AmpliTaq DNA polymerase. Primer extension reactions were placed in the thermal cycler and subjected to one cycle of heating and cooling as follows: 94°C for 2 minutes, 60°C for 2 minutes, 72°C for 5 minutes.

Please replace the paragraph beginning on page 56, line 14 with the following paragraph:

The post-amplification and concerted methods of labeling were compared in the detection of the Factor V Leiden mutation. In the case of post-amplification labeling, the LLA reaction contained 10 pmoles of each primer in the Factor V 18 primer set, template DNA, 0.2 mM dNTPs, 10 mM Tris-HCl pH 9.2, 50 mM KCl, 2.5 mM  $MgCl_2$ , and 2 U AmpliTaq DNA polymerase (Perkin-Elmer) in a total volume of 50  $\mu$ l. Reactions were placed in a thermal cycler (Perkin-Elmer 9600), heated at 94°C for 2 minutes,

then subjected to 35 cycles of heating and cooling as follows:  
94°C, 45 seconds; 50°C, 30 seconds; 72°C, 30 seconds. Finally,  
the reactions were heated at 72°C for 5 minutes. Amplification  
products were labeled by primer extension using 10 pmoles of 5'-  
biotinylated FVU0.1 (5' CTAATCTGTAAGAGCAGATCCCXGGA 3'; SEQ ID  
NO.:60) and 10 pmoles of 5'-biotinylated FVL0.1 (5'  
TTCTGAAAGGTTACTTCAAGGAXAAA 3'; SEQ ID NO.: 61) as primers.

Please replace the paragraph beginning on page 59, line 3 with the  
following paragraph:

LLA primers containing the non-replicable propanediol  
element ("X") were synthesized. These primers correspond to the  
human  $\beta$ -globin cDNA sequence (Genbank Accession No. NM\_000518).  
Primer sequences (5' -> 3') are as follows:

UP1	AGCAACCTCAAACAGACACCAXGGT	SEQ ID NO.:62
UP2.1	TTTGCTTCTGACACAACTGTGTTCACXAGC	SEQ ID NO.:63
UP9	TACCCTTGGACCCAGAGGTTCTTG	SEQ ID NO.:64
UP10	AGTCCTTTGGGGATCTGTCCXCTC	SEQ ID NO.:65
UP11	GCTGTTATGGGCAACCCTAAXGTG	SEQ ID NO.:66
UP12.1	AAGGCTCATGGCAAGAAAGXGCT	SEQ ID NO.:67
UP15	GCACCTGACTCCTGAGGAGAAXTCT	SEQ ID NO.:68
UP16	CCGTTACTGCCCTGTGGGXCAA	SEQ ID NO.:69

UP17	AACGTGGATGAAGTTGGTGGTXAGG	SEQ ID NO.:70
LP25	CACAGTGCAGCTCACTCAGTGXGGC	SEQ ID NO.:71
LP26	TCTCAGGATCCACGTGCAGCXTGT	SEQ ID NO.:72
LP27	AGACCAGCACGTTGCCCAXGAG	SEQ ID NO.:73
LP28	CCAAAGTGATGGGCCAGCXAC	SEQ ID NO.:74
LP29	GCACTGGTGGGGTGAATTCXTTG	SEQ ID NO.:75
LP30	GCCACCACTTTCTGATAGGCAXCCT	SEQ ID NO.:76
LP31	TGTGGGCCAGGGCATTAGXCAC	SEQ ID NO.:77

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Please replace the paragraph beginning on page 60, line 2 with the following paragraph:

First-strand cDNA was prepared by reverse transcription using a propanediol-substituted oligonucleotide  $\beta$ RT1 primer (TGGACAGCAAGAAAGCGAGCXTAG; SEQ ID NO.:78) and the Superscript<sup>TM</sup> Preamplification System (Life Technologies). One (1)  $\mu$ l of whole blood total RNA (approximately 1  $\mu$ g), 1  $\mu$ l of 10  $\mu$ M  $\beta$ RT1 primer and 10  $\mu$ l of water were mixed and heated at 70°C for 10 minutes, then at 55°C for 1 minute. After the addition of four  $\mu$ l of 5X First Strand Buffer (1X buffer contains 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl<sub>2</sub>), 1  $\mu$ l of 10 mM dNTPs and 2  $\mu$ l of 0.1 M dithiothreitol, the sample was heated at 55°C for 1

minute. One (1)  $\mu$ l of Superscript II reverse transcriptase (200 Units) was then added and the mixture incubated sequentially at 55°C for 30 minutes, 70°C for 15 minutes and 37°C for 1 minute. Finally, 0.5  $\mu$ l of RNase H (1 Unit) was added and the mixture heated at 37°C for 20 minutes. The final volume of the cDNA preparation was 20.5  $\mu$ l. As a control, a parallel reaction was carried out using a non-substituted oligonucleotide  $\beta$ RT1-x primer (TGGACAGCAAGAAAGCGAGCTTAG; SEQ ID NO.:79).

Please replace the paragraph beginning on page 60, line 16 with the following paragraph:

In order to assess the efficiency of the priming reaction conducted with the propanediol-substituted oligonucleotide  $\beta$ RT1 primer, PCR reactions were carried out using an aliquot of each first-strand cDNA sample (primed with  $\beta$ RT1 or  $\beta$ RT1-x) and PCR primers UP2-x (TTTGCTTCTGACACAACTGTGTTTCAC; SEQ ID NO.:80) and LP26-x (TCTCAGGATCCACGTGCAGCTTGT; SEQ ID NO.:81). Each PCR reaction contained 10 pmoles of UP2-x; 10 pmoles of LP26-x; the equivalent of 0.08  $\mu$ l, 0.4  $\mu$ l or 2  $\mu$ l of first strand cDNA preparation; 0.2 mM dNTPs; 10 mM Tris-HCl pH 9.2; 50 mM KCl; 2,5 mM MgCl<sub>2</sub>, and 1 U AmpliTaq DNA polymerase in a total volume of 25  $\mu$ l. Samples were placed in the thermal cycler, heated at 94°C

for 2 minutes, then subjected to 17 cycles of heating and cooling as follows: 94°C, 45 seconds; 62°C, 15 seconds; 72°C, 30 seconds. At the end of the last cycle, reactions were heated at 72°C for 4.5 minutes.

Please replace the paragraph beginning on page 62, line 7 with the following paragraph:

Amplification products were labeled in a primer extension reaction using the 5' biotinylated primer biotin-LP24 (AAAGGTGCCCTTGAGGTTGTCCAG; SEQ ID NO.:82). A 10 ul aliquot of the LLA or PCR reaction was mixed with 1 ul of 10 uM biotin-LP24. Samples were placed in the thermal cycler and subjected to one cycle of heating and cooling as follows: 94°C, 2 minutes; 55°C, 2 minutes; 72°C, 5 minutes.

Please replace the paragraph beginning on page 62, line 13 with the following paragraph:

Biotin-labeled DNA fragments were detected colorimetrically after capture in microwells coated with oligonucleotide 010599 (CCTTTAGTGATGGCCTGGCT; SEQ ID NO.:83). Detection reagents used were components of the Bio-Rad mDx Variant Gene 1 kit. The primer extension-labeling reaction (11

ul) was mixed with 10 ul of Denaturation Solution then incubated for 10 minutes at room temperature. A 10 ul aliquot of the denatured sample and 40 ul of hybridization solution were loaded into a well containing immobilized oligonucleotide 010599. The well was incubated for 1 hour at 37°C then washed five times with Well Wash Buffer. Then, 50 ul of SA-HRP (streptavidin-horseradish peroxidase conjugate) were added to the well. The well was incubated for 30 min. at 37°C then washed five times with Well Wash Buffer. Next, 50 ul of Substrate TMB (tetramethyl benzidine) were added to the well. After 10 minutes at room temperature, the colorimetric reaction was stopped by adding 50 ul of Stop Solution. Absorbance was read at 450 nm with 595 nm as reference wavelength.

IN THE CLAIMS

1. (Amended) A process for generating a complementary DNA (cDNA) molecule from an RNA molecule comprising:
  - a) annealing a first primer containing a non-replicable element, with or without a cleavable element, to an RNA molecule,
  - b) generating a first strand cDNA product,



- c) annealing a second primer containing a non-replicable element, with or without a cleavable element, to the first strand cDNA product, and
- d) generating a second strand cDNA product that is a complement of said first cDNA strand.

2. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 1 ;
- b) combining [the] said first and [second] said complement cDNA strands in a reaction mixture with a primer containing a non-replicable element and/or a cleavable element, under conditions such that first generation primer extension products are produced using said strands as templates, and wherein [the] said primer for [the first] a cDNA strand is selected such that a first generation primer extension product produced using [the first] said cDNA strand as a template, when separated from [the first] said cDNA strand, can serve as a template for synthesis of a second generation primer extension

product of the primer for [the second] said  
complement strand;

- c) separating [the] said first generation primer extension products; from their respective templates to produce single-stranded molecules; and
- d) treating the first generation primer extension products with the primers of claim 2, step (b) under conditions such that second generation primer extension products are produced using the first generation primer extension products as templates, wherein the second-generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of [interest] said first generation primer extension product and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products of claim 2.

3. (Amended) A process for amplifying a cDNA molecule comprising:

- (a) generating a cDNA molecule according to the process of claim 1,

- (b) combining [the] said first and [second] said complement cDNA strands in a reaction mixture with first primers and second primers, each of [said first and second primers] which contain[ing]s a non-replicable element and/or a cleavable element, under conditions such that a first generation primer extension product is synthesized using said strands as templates, and wherein the first and second primers are selected such that the first generation primer extension products, when separated from their templates, can serve as templates for synthesis of second generation primer extension products of the first and second primers;
- (c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- (d) treating the first generation primer extension products with the first and second primers under conditions such that second generation primer extension products are synthesized using the first generation primer extension products as templates, wherein the second-generation primer extension

products contain at least a portion of the sequence of the nucleic acid sequence of [interest] said first generation primer extension product and no more than an insufficient portion of the binding site for said first and second primers for producing said first-generation primer extension products.

4. (Amended) A process for amplifying a cDNA molecule comprising:

- (a) generating a cDNA molecule according to the process of claim 1 ;
- (b) combining [the], said first and [second] said complement cDNA strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and are 5' to one another with respect to said strand and which do not overlap with one another at the position of said non-replicable element or cleavable element;

- (c) subjecting said reaction mixture to conditions such that first generation primer extension products are produced from first nested primers, and not from other of said series of nested primers using the first and second strands as templates, wherein said first nested primers are primers of, said nested primers which are most 3' with respect to said sequence of [interest] said first and second cDNA strands , and wherein the first nested primers are selected such that a first generation primer extension product [from this step], when separated from its template, can serve as a template for synthesis of a second generation extension product of the first nested primer for the complement [of said] strand;
- (d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules;
- (e) exposing said reaction mixture to conditions such that second generation primer extension products are generated by said first nested primers using first generation primer extension products as

templates, wherein the second generation primer extension products contain at least portion of the sequence of the nucleic acid sequence of [interest] said first generation primer extension product and no more than an insufficient portion of the binding site for said first nested primers for producing said first generation primer extension products;

- (f) separating the second generation primer extension products from their template to produce single stranded molecules;
- (g) subjecting the reaction mixture of step (f) to reaction conditions such that next generation primer extension products are synthesized from another nested primer of said series of nested primers using second generation primer extension products as templates, and separating the thus produced next generation primer extension products from their templates to produce single-stranded molecules; and
- (h) repeating step (g) such that each repeat of [the] step (g) comprises subjecting the reaction

mixture to conditions such that next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.

5. (Amended) A process for amplifying a cDNA molecule comprising:

- (a) generating a cDNA molecule according to the process of claim[s] 1;
- (b) combining [the;] said first and [second] said complement strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and flank the sequence of interest but do not overlap with one another at the position of said non-replicable element or cleavable element;
- (c) subjecting said reaction mixture to conditions whereby each of said nested primers is capable of binding to its respective complementary site;

- (d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
  - (e) repeating steps (c) and (d) whereby next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.
8. (Amended) The process of anyone of claims 2-5, wherein said primer of step (b) of the dependent claim contains a non-replicable element.
9. (Amended) The process of anyone of claims 2-5, wherein said primer of step (b) of the dependent claim contains a cleavable element.
16. (Amended) A process for generating a complementary DNA (cDNA) molecule from an RNA molecule comprising:
- a) annealing a primer containing a non-replicable element, with or without a cleavable element, to an RNA molecule, and
  - b) generating a first strand cDNA product.



17. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 16;
- b) combining the cDNA strand in a reaction mixture with a primer containing a non-replicable element and/or a cleavable element, under conditions such that first-generation primer extension product[s are] is produced using said strand[s] as a template[s], and wherein the primer for [the first] said strand is selected such that a first generation primer extension product produced using [the first] said strand as a template, when separated from [the first] said strand, can serve as a template for synthesis of a second generation primer extension product of [the] a primer for the [second strand] first generation primer extension product;
- c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- d) treating the first generation primer extension products with the primers [of] described in step (b ) under

conditions such that second generation primer extension products are produced using the first generation primer extension

products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of [interest] the first generation primer extension product and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products.

18. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 16;
- b) combining the first and second cDNA strands in a reaction mixture with first primers and second primers, each of said first and second primers containing a non-replicable element and/or a cleavable element, under conditions such that a first generation primer extension product is synthesized using said strands as templates, and wherein the first and second primers are selected such that the first generation primer extension

products, when separated from their templates, can serve as templates for synthesis of second generation primer extension products of the first and second primers;

- c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- d) treating the first generation primer extension products with the first and second primers under conditions such that second generation primer extension products are synthesized using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of [interest] the first generation primer extension product and no more than an insufficient portion of the binding site for said first and second primers for producing said first generation primer extension products.

19. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 16;

- b) combining the first and second cDNA strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and are 5' to one another with respect to said strands and which do not overlap with one another at the position of said non-replicable element or cleavable element;
- c) subjecting said reaction mixture to conditions such that first generation primer extension products are produced from first nested primers, and not from other of said series of nested primers using the first and second strands as templates, wherein said first nested primers are primers of said nested primers which are most 3' with respect to said sequence [of interest], and wherein the first nested primers are selected such that a first generation primer extension product [from this step], when separated from its template, can serve as a template for synthesis of a second generation extension product of the first nested primer for the complement [of said] strand;

- d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules;
- e) exposing said reaction mixture to conditions such that second generation primer extension products are generated by said first nested primers using first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of [interest] the first generation primer extension product and no more than an insufficient portion of the binding site for said first nested primers for producing said first generation primer extension products;
- f) separating the second generation primer extension products from their template to produce single stranded molecules;
- g) subjecting the reaction mixture of step (f) to reaction conditions such that next generation primer extension products are synthesized from another nested primer of said series of nested primers using second generation primer extension products as templates, and separating

- c) subjecting said reaction mixture to conditions whereby each of said nested primers is capable of binding to its respective complementary site;
  - d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
  - e) repeating steps (c) and (d) whereby next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.
23. (Amended) The process of anyone of claims 17 -20, wherein said primer of step (b) contains a non-replicable element.
24. (Amended) The process of any one of claims 17 -20, wherein said primer of step (b) contains a cleavable element.

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